Supplementary Materials

Supplementary Methods

1. Procedures of diagnosis.

Criteria for MCI were retrospectively applied among the non-dementia individuals after the conference. Consistent with the standard criteria, for all subtypes of MCI described below, the subjects considered to have MCI were required to have (1) objective impairment in one cognitive domain based on the average of scores on neuropsychological measures within that domain, and 1 SD and 1.5 SD cutoffs derived from normative corrections for age, years of education, and sex, (2) essentially preserved activities of daily living, (3) presence of memory complaints and (4) no diagnosis of dementia by group consensus.

First, for our subtype of amnestic (a)MCI single, memory impairment was defined as a score <1 or 1.5 SD below the demographically corrected mean on the category-cued recall test; performance scores from all other cognitive domains were required to fall within normal limits (>1 or 1.5 SD below the demographically corrected mean). Second, aMCI multiple was diagnosed in the presence of memory impairment in one or more cognitive domains. Third, a diagnosis of non-amnestic (na)MCI single required cognitive impairment in a single non-memory domain and normal performance scores in all other cognitive domains. Finally, naMCI multiple was diagnosed if impairment was seen in two of the four non-memory domains and when the memory domain score was within normal limits.

2. MRI

Each processed segmented image was compared to the mean and standard deviation of gray matter or white matter images of 80 healthy controls (40 males and 40 females, aged 54-86 years). The severity of atrophic changes was calculated by the z-score. The following equation was used: z-score = ([control mean]–[individual value])/(control standard deviation). One voxel was defined as 2 mm × 2 mm × 2 mm.

3. Measurement of cholesterol, glucose, and HbA1c

Low-density lipoprotein (LDL), high-density lipoprotein (HDL), total cholesterol (TC), and triglyceride levels were measured by enzymatic methods using commercial kits (Sekisui Medical, Tokyo, Japan). Blood glucose and HbA1c were also measured by enzymatic methods using

Serotec GLU-L (Serotec, Sapporo, Japan) and CinQ HbA1c (LSI Medience Corp., Tokyo, Japan), respectively. The *APOE* genotype was determined as follows: after 40 cycle-amplification, the polymerase chain reaction product was digested with the restriction enzyme *Hha*I, and subjected to electrophoresis in a 10% polyacrylamide gel [1].

4. Immunohistochemistry of complement proteins and Aβ 42 in human brain specimens

Hippocampal tissue sections were deparaffinized and rehydrated, and antigen retrieval was performed by treatment of the slides in 0.01 M citrate buffer (pH 6.0) for 10 min in the autoclave. Thereafter, the slides were cooled to 22 ± 2°C and washed in phosphate buffered saline. Slides were immersed in 1% hydrogen peroxide for 45 min to block endogenous peroxidase activity. After washing and blocking, the sections were incubated at 4ºC overnight with anti-C3 antibody, followed by a standard procedure using a Histofine MAX-PO secondary antibody (Nichirei, Tokyo, Japan) and DAB substrate kit (Nichirei). In senile plaque staining, treatment with formic acid was performed instead of autoclave treatment.

5. Statistical analysis

The area under the curve (AUC) of the receiver operating characteristic (ROC) was calculated by MedCalc (ver. 9.3.9; MedCalc Software, Mariakerke, Belgium). The closest point to the upper left corner of the ROC curve provided the optimum sensitivity and specificity values. Multivariate linear regression was used to analyze the relationship between the three sequester protein levels and the severity of cognitive decline, and can be used to model the relationship between one or more variables by fitting a linear equation. The least absolute shrinkage and selection operator (LASSO) modeling using the glmnet package (ver. 1.9-5) for R (ver. 3.1.0; R Foundation for Statistical Computing, Vienna, Austria) was used to evaluate the combination of multiple biomarkers. The results of the linear equation were further analyzed by *C*-statistics

References

[1] Wenham PR, Price WH, Blandell G. Apolipoprotein E genotyping by one-stage PCR. Lancet 1991;337:1158-1159.

Supplementary Table 1. Seum biomarker levels in APOE ε4- and ε4+ participants

Characteristics	APOE4 - (n = 210)	APOE4 + (n = 63)	*P* value*
Age	69.6 ± 11.5[†]	69.6 ± 11.5	0.59444
MMSE score	27.0 ± 4.3	27.4 ± 4.0	0.72149
aC3, mg/dL	99.5 ± 17.6	103.0 ± 16.5	0.20594
nC3, unit/mL	1.2 ± 0.7	0.2 ± 0.7	0.92173
aC3/nC3	103.6 ± 48.7	105.7 ± 41.3	0.4171
C4, mg/dL	24.6 ± 5.7	25.6 ± 6.1	0.31319
TTR, mg/dL	24.3 ± 6.1	24.7 ± 6.5	0.50545
ApoA-I, mg/dL	156.6 ± 28.5	147.1 ± 26.0	0.01635
ApoE, mg/dL	4.1 ± 1.2	3.7 ± 1.0	0.01945
HDL, mg/dL	64.0 ± 17.7	59.0 ± 17.0	0.05719
LDL, mg/dL	120.1 ± 30.8	125.7 ± 32.9	0.16833
TC, mg/dL	200.0 ± 33.9	199.4 ± 37.5	0.87752
TG, mg/dL	155.2 ± 83.5	149.9 ± 92.2	0.42018
BS, mg/dL	120.2 ± 38.1	116.5 ± 28.5	0.77508
HbA1c (%)	5.7 ± 0.6	5.7 ± 0.5	0.79484
Triple-marker sore (ApoA-I,TTR, nC3)	0.62 ± 0.29	0.71 ± 0.27	0.0091
Triple-marker sore (ApoA-I,TTR, aC3/nC3)	0.67 ± 0.25	0.74 ± 0.21	0.0186

*Mann-Whitney test. Significant differences among 2 groups are indicated.

[†]mean ± SD

Abbreviations: aC3, active form of C3; nC3, native form of C3; TTR, transthyretin; TC, total cholesterol; TG, triglyceride; BS, blood sugar.

Supplementary Table 2. Association of serum levels of TTR, apoA-I, HDL, and TC with MMSE score

Characteristics	27-30 (n = 208)	24-26 (n = 26)	20-23 (n = 20)	<20 (n = 19)	*P* value*
Age	66.2 ± 9.4†	72.7 ± 9.2	83.6 ± 5.6	84.2 ± 9.5	3.76E-16
Male/Female	79 / 129	11 / 15	5 / 15	8 / 11	
APOE4 genotype	54 (26%)	3 (11.5%)	1 (5%)	5 (26.3%)	
aC3, mg/dL	100.2 ± 18.1	98.6 ± 14.6	106.7 ± 14.5	97.4 ± 14.8	0.30123
nC3, unit/mL	1.2 ± 0.7	1.2 ± 0.6	1.6 ± 1.0	1.3 ± 0.7	0.11524
aC3/nC3	105.3 ± 44.8	108.1 ± 51.9	94.1 ± 59.3	95.9 ± 52.2	0.31808
C4, mg/dL	24.8 ± 6.0	25.9 ± 5.5	22.4 ± 5.2	26.0 ± 4.7	0.10209
TTR, mg/dL	25.2 ± 5.9	24.9 ± 5.2	21.6 ± 7.1	18.2 ± 6.3	5.75E-05
ApoA-I, mg/dL	157.2 ± 27.5	154.8 ± 26.2	145.2 ± 28.9	132.6 ± 27.7	0.00345
ApoE, mg/dL	4.0 ± 1.1	3.9 ± 1.0	4.0 ± 1.0	3.6 ± 0.9	0.39706
HDL, mg/dL	65.1 ± 17.7	61.6 ± 15.6	53.8 ± 14.0	51.8 ± 15.9	0.00134
LDL, mg/dL	122.9 ± 33.0	126.0 ± 22.1	120.0 ± 28.4	104.4 ± 23.3	0.05119
TC, mg/dL	203.9 ± 34.8	204.3 ± 26.8	191.3 ± 31.0	166.9 ± 27.5	8.77E-05
TG, mg/dL	156.8 ± 89.0	157.3 ± 74.5	160.6 ± 93.7	117.6 ± 46.3	0.37443
BS, mg/dL	118.4 ± 30.8	115.7 ± 31.9	144.1 ± 78.0	112.6 ± 33.8	0.52723
HbA1c (%)	5.6 ± 0.5	5.7 ± 0.6	6.0 ± 0.9	5.7 ± 0.8	0.27679
Triple-marker sore (ApoA-I,TTR, nC3)	0.65 ± 0.29	0.67 ± 0.27	0.55 ± 0.31	0.68 ± 0.26	0.62755
Triple-marker sore (ApoA-I,TTR, aC3/nC3)	0.69 ± 0.25	0.71 ± 0.25	0.67 ± 0.21	0.72 ± 0.21	0.96442

*Kruskal-Wallis test. Significant differences among 4 groups are indicated.

†mean ± SD

Abbreviations: aC3, active form of C3; nC3, native form of C3; TTR, transthyretin; TC, total cholesterol; TG, triglyceride; BS, blood sugar.

Supplementary Table 3. Correlations between serum biomarker levels and age or MMSE score

	Age		MMSE score	
Characteristics	coefficient r	*P* value*	coefficient r	*P* value*
aC3, mg/dL	0.01519	0.8027	0.04241	0.48533
nC3, unit/mL	0.16312	0.00691	-0.10817	0.07436
aC3/nC3	-0.07407	0.22247	0.06428	0.2899
C4, mg/dL	0.07417	0.26686	-0.08077	0.22648
TTR, mg/dL	-0.4074	2.44E-12	0.30449	2.89E-07
ApoA-I, mg/dL	-0.19377	0.00129	0.24329	4.86E-05
ApoE, mg/dL	-0.0584	0.38223	0.10353	0.12151
HDL, mg/dL	-0.19266	0.00364	0.26565	5.25E-05
LDL, mg/dL	-0.19136	0.00388	0.14556	0.02869
TC, mg/dL	-0.29534	6.29E-06	0.29819	5.06E-06
TG, mg/dL	-0.13317	0.04552	0.10241	0.12476
BS, mg/dL	0.13874	0.02584	-0.03621	0.56257
HbA1c (%)	0.14567	0.01601	-0.09165	0.13089
Triple-marker sore (ApoA-I,TTR, nC3)	-0.11306	0.06163	0.01557	0.79746
Triple-marker sore (ApoA-I,TTR,	-0.05276	0.38522	-0.02758	0.65

*Pearson test. Significant correlation between serum biomarker levels and age or MMSE score are indicated.

Abbreviations: aC3, active form of C3; nC3, native form of C3; TTR, transthyretin; TC, total cholesterol; TG, triglyceride; BS, blood sugar.

Supplementary Table 4. Serum levels of proteins involved in amyloid-β clearance and choresterols in participants categorized by VSRAD score in MRI

	VSRAD 0-1 (n = 27)	VSRAD 1-2 (n = 25)	VSRAD >2 (n = 9)	*P* value*
Age	63.2 ± 7.8[†]	68.1 ± 10.7	76.1 ± 7.8[‡§]	0.00374
Male/Female	12 / 15	9 / 16	2 / 7	
APOE e4 carrier, %	25.9	28.0	33.3	
aC3, mg/dL	98.7 ± 15.9	103.7 ± 15.6	102.8 ± 20.0	0.38068
nC3, unit/mL	1.0 ± 0.3	1.1 ± 0.5	1.3 ± 0.5	0.39886
aC3/nC3	103.7 ± 32.1	113.6 ± 44.3	97.0 ± 50.5	0.52451
C4, mg/dL	23.7 ± 4.4	28.5 ± 4.9[¶]	26.6 ± 6.9	0.01239
TTR, mg/dL	24.7 ± 6.2	25.4 ± 5.2	21.7 ± 4.1	0.17529
ApoA-I, mg/dL	164.3 ± 30.4	150.3 ± 21.7	133.9 ± 24.9[‡]	0.01801
ApoE, mg/dL	3.8 ± 0.7	4.4 ± 0.7	4.4 ± 1.2	0.13135
HDL, mg/dL	68.3 ± 20.2	62.0 ± 15.1	53.0 ± 19.5	0.09237
LDL, mg/dL	119.0 ± 31.8	134.1 ± 31.9	120.7 ± 24.4	0.45973
TC, mg/dL	200.4 ± 31.5	210.2 ± 35.1	194.6 ± 27.1	0.80258
TG, mg/dL	135.1 ± 58.1	147.6 ± 77.3	168.0 ± 114.6	0.9444
BS, mg/dL	112.8 ± 30.5	124.8 ± 41.4	111.2 ± 17.4	0.42675
HbA1c (%)	5.6 ± 0.4	5.8 ± 0.6	5.8 ± 0.8	0.78512
Triple-marker sore (ApoA-I,TTR, nC3)	0.63 ± 0.27	0.73 ± 0.27	0.74 ± 0.28	0.40761
Triple-marker sore (ApoA-I,TTR, aC3/nC3)	0.63 ± 0.26	0.77 ± 0.27	0.76 ± 0.28	0.19772

*Kruskal-Wallis test. Significant differences among 3 groups are indicated.

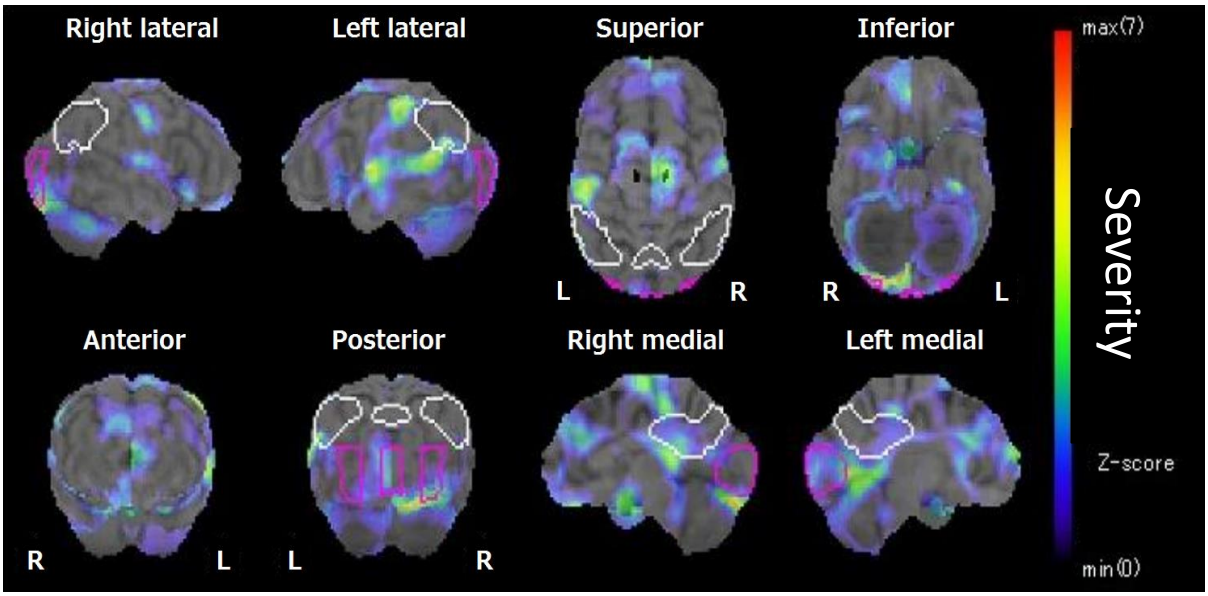
[†]mean ± SD

[‡]Holm-Bonferroni test. Significant differences in VSRAD 0-1 vs. VSRAD >2 were observed in age (*P* = 4.9E-04) and apoA-I (*P* = 0.00402).

[§]Holm-Bonferroni test. Significant differences in VSRAD >2 vs. VSRAD 1-2 were observed in age (P = 0.02757).

[¶]Holm-Bonferroni test. Significant differences in VSRAD 0-1 vs. VSRAD 1-2 were observed in C4 (P = 0.00403).

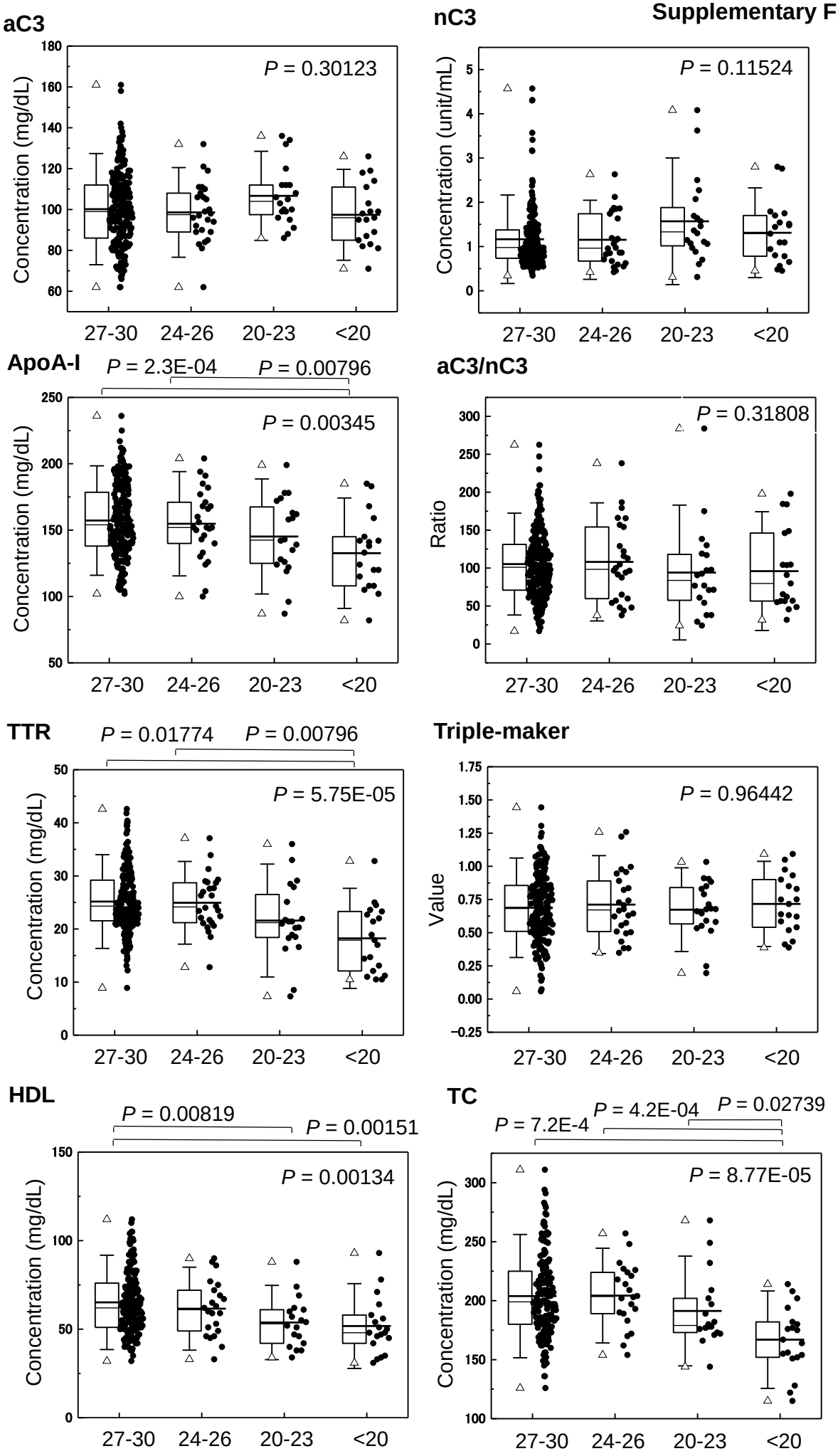
Abbreviations: aC3, active form of C3; nC3, native form of C3; TTR, transthyretin; TC, total cholesterol; TG, triglyceride; BS, blood sugar.



Supplementary Figure 1. Assessment of reduction of regional cerebral blood flow

Automatic statistical analysis of brain perfusion was conducted using SPECT data. The subjects were categorized into four groups by reduction of rCBF as follows; **A**: No abnormality in four ROIs including the left/right posterior cingulate gyrus/precuneus and parietal cortex. **B**: Decreased rCBF in one ROI. **C**: Decreased rCBF in two ROIs and decreased rCBF in the posterior cingulate gyrus/precuneus. **D**: Decreased rCBF in three ROIs and decreased rCBF in the posterior cingulate gyrus and precuneus. Abbreviations: rCBF, regional cerebral blood flow; ROI, region of interest.

Supplementary Figure 2



Supplementary Figure 2. Serum levels of the proteins involved in Aβ clearance according to the MMSE scores

Participants were grouped according to MMSE scores: 27-30 (n = 208), 24-27 (n = 26), 20-23 (n = 20), and <20 (n = 19). Open triangles indicate the highest and lowest values in each group. Error bars represent ± 1.5 standard deviation. Significant differences among the three groups are indicated (Kruskal-Wallis test). Significant differences in protein levels between two groups are also indicated (Bonferroni test).